Basic and Clinical
NEUROSCIENCE
October 2014 . Volume 5. Number 4

Brain Tissues Oxidative Damage as A Possible Mechanism of Deleterious Effects of Propylthiouracil- Induced Hypothyroidism on Learning and Memory in Neonatal and Juvenile Growth in Rats

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Article info:
Received: 04 August 2013
First Revision: 01 February 2014
Accepted: 22 March 2014

Key Words:
Hypothyroidism,
Lactation, Neonatal,
Juvenile, Learning,
Memory, Oxidative stress

ABSTRACT

Introduction: The role of brain tissues oxidative damage in learning and memory impairments has been well documented. It is also well known that thyroid hormones have a critical role for the brain functions. The purpose of this study was to investigate the role of brain tissues oxidative damage as a possible mechanism of deleterious effects of propylthiouracil (PTU) - induced hypothyroidism on learning and memory in neonatal and juvenile growth in rats.

Methods: Fourteen pregnant female Wistar rats were kept in separate cages. After delivery, they were randomly divided into two groups including control and PTU. Rats in the control group received normal drinking water, whereas the second group received drinking water supplemented with 0.02% PTU from the first day after delivery through the first two months of the life of offspring (the pups of rats). After 60 days, nine male offspring of each group were randomly selected and tested in the Morris water maze (MWM). Then, samples of blood were collected to measure thyroxine. Finally, the brains were removed and total thiol groups and molondialdehyde (MDA) concentrations were determined.

Results: Compared to the control group's offspring, serum thyroxine levels in the PTU group's off spring were significantly low ($P<0.001$). In MWM, the escape latency and traveled path in the PTU group were significantly higher than that in the control group ($P<0.01$- $P<0.001$). In PTU group, the total thiol concentrations in both cortical and hippocampal tissues were significantly lower and MDA concentrations were higher than control group ($P<0.001$).

Discussion: It seems that deleterious effect of hypothyroidism during neonatal and juvenile growth on learning and memory is at least in part due to brain tissues oxidative damage.

1. Introduction

Thyroid hormones are known to set the cellular basal metabolic rate and are considered as major regulators of energy metabolism, mitochondrial activity, oxygen consumption and active oxygen metabolism (Katyare, Bangur, & Howland, 1994; Martinez et al., 2001; Weitzel & Iwen, 2003). Thyroid hormones also play a crucial role in new neuron production (Shin et al., 2013). It has been shown that hypothyroidism produced experimentally in neonatal rats lead to structural defects in the nervous system which is accompanied with behavioral abnormalities (Cragg, 1970; Eayrs & Horn, 1955; Eayrs & Taylor, 1951). Severe cognitive and neurological impairments due to deficiency of thyroid hormones during brain development have been well documented (Desouza et al., 2005). Prenatal deficiency of thyroid hormones impairs learning ability and memory function because of decreased neuronal survival, attenuated

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dendritic spine density, and disturbed synaptic function (Gong et al., 2010; Rivas & Naranjo, 2007; Sala-Roca, Estebanez-Perpina, Balada, Garau & Marti-Carbonell, 2008; Shibutani et al., 2009). Other evidence has shown that maternal hypothyroidism during lactation lead to incomplete development of the brain in their offspring (Morreale de Escobar, Obregon & Escobar del Rey, 2004). Hypothyroidism even in adulthood has also been clearly linked to cognitive dysfunctions, disturbed attention and depressed moods (Dugbartey, 1998; Jackson, 1998). Thyroid status in adults affects morphologically those regions of the brain that are strongly involved in learning, memory and mood, such as the hippocampus (Desouza et al., 2005). A reduced number of neurons in the hippocampus of adult hypothyroid rats was also reported (Alva-Sanchez, Ortiz-Butron, & Pacheco-Rosado, 2004; Alva-Sanchez et al., 2009). Hippocampus was reported to be smaller in hypothyroid rats in comparison with normal ones (Rabie, Patel, Clavel & Legrand, 1979).

In contrast with these findings, other reports suggested the preventive effects of hypothyroidism against neuronal death induced by transient cerebral and focal ischemia (Rastogi et al., 2006; Shuaib et al., 1994; Shuaib et al., 1994) probably by reducing the oxidative stress (Tenorio-Velazquez et al., 2005). It was also reported that hypothyroid state postponed neuronal death in CA1 area of hippocampus after transient cerebral ischemia by reducing lipid peroxidation and increasing superoxide dismutase (Lee et al., 2010). In contrast, it has been reported that thyroid hormones have antioxidant properties (Ahmed, Ahmed, El-Gareib, El-Bakry, & Abd El-Tawab, 2012). Hypothyroidism also leads to selectively oxidative stress in the hippocampus and amygdala (Cano-Europa et al., 2008). Yet, other studies have confirmed the oxidative stress followed by hypothyroidism (Konukoglu, Ercan, & Hatemi, 2002; Yilma, Ozan, Benzer & Canatan, 2003).

The present study aimed to investigate the role of brain tissues oxidative damage as a possible mechanism for deleterious effects of propylthiouracil (PTU)- induced hypothyroidism on learning and memory in neonatal and juvenile rats.

Total thiols are extraordinarily efficient antioxidants with the ability to react with free radicals (Sen, 1998; Wlodek, 2002) and are known to be sensitive to oxidative damage and depleted following an oxidative insult (Soszynski & Bartosz, 1997). Therefore, we studied the effect of hypothyroidism on total thiol concentrations in brain tissues.

2. Methods

2.1. Animals and Treatments

Fourteen pregnant female Wistar rats (12 weeks old and weighing 220 - 250 g) were kept in separate cages at 22 ± 2 °C in a room with a 12 h light/dark cycle (light on at 7:00 am). They were randomly divided into two groups and treated according to the experimental protocol from the first day after delivery through the first two months of the life of offspring (the pups of the rats). Rats in the control group received normal drinking water, whereas the second group received the same drinking water supplemented with 0.02% PTU (Sigma, USA) to induce hypothyroidism. After 60 days, nine male offspring of each group were randomly selected and tested in the Morris water maze (MWM). The number of animals in each group was based on our previous studies (Hosseini et al., 2010). In the PTU group, hypothyroidism was confirmed by testing serum thyroxine concentration levels using the radioimmunoassay method. Animal handling and all related procedures were carried out in accordance with the rules set by Ethical Committee of Mashhad University of Medical Sciences, Mashhad, Iran.

2.2. Morris Water Maze Apparatus and Procedures

A circular black pool (136 cm diameter, 60 cm high, 30 cm deep) was filled with water (24–26 °C). A circular platform (10 cm diameter, 28 cm high) was placed within the pool and was submerged approximately 2 cm below the surface of the water in the center of the southwest quadrant. Outside the maze, fixed visual cues were presented at various locations around the room (i.e. a computer, hardware and posters). Before each experiment, each rat was handled daily for 3 days and habituated to the water maze for 30 sec without a platform. The animals performed four trials on each of the eight consecutive days, and each trial began with the rat being placed in the pool and released facing the side wall at one of four positions (the boundaries of the four quadrants, labeled as North (N), East (E), South (S) and West (W). Release positions were randomly predetermined. For each trial, the rat was allowed to swim until it found and remained on the platform for 15 seconds. If 60 seconds had passed and the animal had not found the platform, it was guided to the platform by the experimenter and allowed to stay on the platform for 15 sec. Then, the rat was removed from the pool, dried and placed in its holding bin for 5 min. The time latency to reach the platform and the length of the swimming path were recorded by a video tracking system (Hosseini et al., 2010; Saffarzadeh

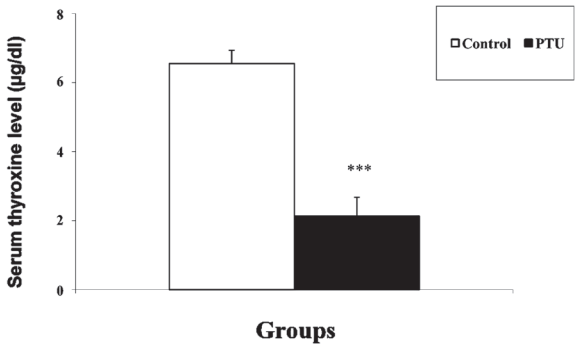

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Figure 1. Serum thyroxine concentrations in offspring of PTU and control groups. Control group received tap drinking water, the animals of other group received PTU (0.02%) in drinking water. Data are shown as mean ± SEM of 9 animals in each group. ***P<0.001 compared to control.

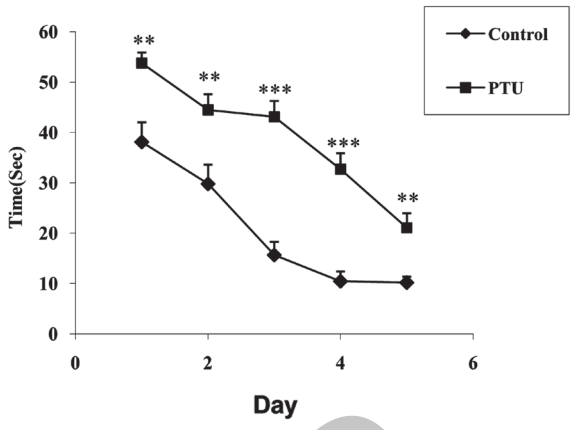

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Figure 2. Comparison of the time latency (sec) between offspring of PTU and control groups. The time latency was significantly higher in offspring rats of PTU group compared to the control group (P<0.001). Control group received tap drinking water, the animals of other group received PTU (0.02%) in drinking water. Data are shown as mean ± SEM of 9 animals in each group. **P<0.01, ***P<0.001 compared to control.

et al., 2010). All measurements were performed during the second half of the light cycle.

2.3. Biochemical Assessment

After the last session of the MWM test, blood samples were taken from all rats to determine hypothyroidism status. Finally, the animals were sacrificed and the cortical and hippocampal tissues were removed, weighed and submitted to determination of total thiol (SH) groups and malondialdehyde (MDA) concentrations. Total SH groups were measured using DTNB (2, 2'-dinitro- 5, 5'-dithiodibenzoic acid) as the reagent.

This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm. Briefly, 1 ml Tris-EDTA buffer (pH=8.6) was added to 50 µl brain homogenate in 1 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 µl DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min (stored in laboratory temperature), the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B).

Total thiol concentration (mM) was calculated by the following equation (Hosseini, Pourganji, Khodabandehloo, Soukhtanloo & Zabihi, 2012; Khodabandehloo et al., 2013).

Total thiol concentration (mM) = (A2-A1-B) × 1.07/0.05 × 13.6

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 535 nm. 2 ml from reagent of TBA/TCA/HCL was added to 1 ml of homogenate and the solution was heated in a water bath for 40 min. After cooling, the whole solutions were centrifuged within 1000g for 10 min. The absorbance was measured at 535 nm (Hosseini, Pourganji, Khodabandehloo, Soukhtanloo & Zabihi, 2012; Khodabandehloo et al., 2013). The MDA concentration was calculated by the following equation:

$$C\ (M) = \text{Absorbance} / (1.56 \times 10^5).$$

2.4. Statistical Analysis

All data were expressed as means ± SEM. The data of time and distance during 5 days of MWM were compared using repeated measures ANOVA test. MDA concentrations and total thiol groups were compared by unpaired student t test. Differences were considered statistically significant when P<0.05.

3. Results

The serum thyroxine concentration in PTU treated animals was significantly low compared to that of control animals (P<0.001; Figure 1).

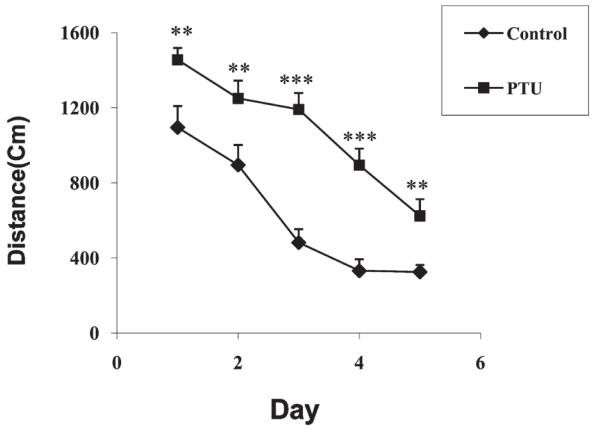

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Figure 3. Comparison of the length of swimming path (cm) between offspring of PTU and control groups using repeated measure ANOVA. The length of swimming path in offspring of PTU group was significantly higher than of control (P<0.001). Control group received tap drinking water, the animals of PTU group received PTU (0.03%) in drinking water. Data are shown as mean ± SEM of 9 animals in each group. **P<0.01, ***P<0.001 compared to control.

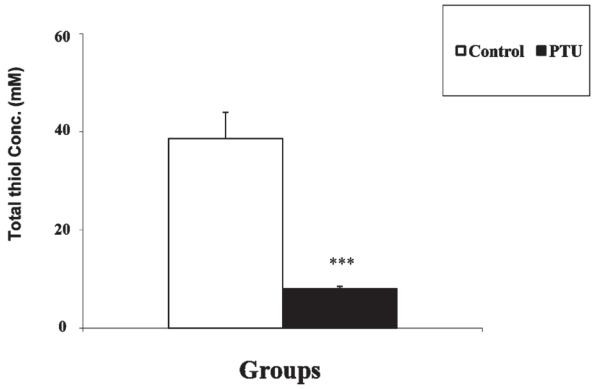

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Figure 4. Comparison of total thiol concentrations in cortical tissues between offspring of PTU and control groups. ***P<0.001 compared to control.

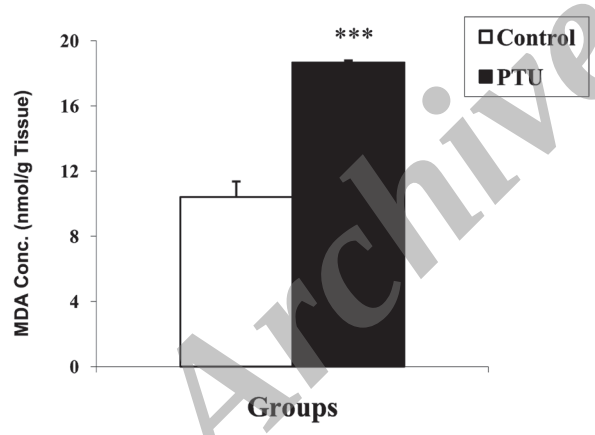

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Figure 5. Comparison of MDA concentrations in cortical tissues between offspring of PTU and control groups. ***P<0.001 compared to control.

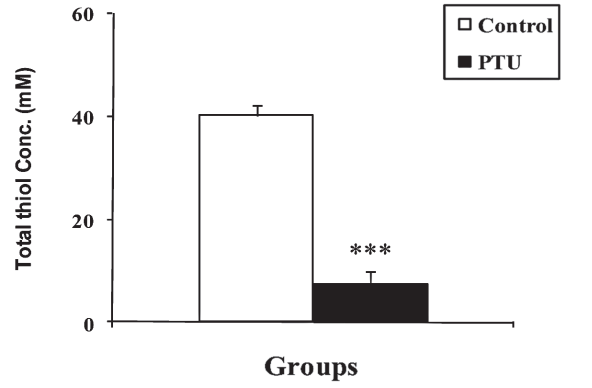

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Figure 6. Comparison of total thiol concentrations in hippocampal tissues between offspring of PTU and control groups. ***P<0.001 compared to control.

Time latency to reach the platform in PTU groups was significantly higher than that Control group in all five days (P<0.01- P<0.001; Figure 2). The animals of PTU group also traveled longer distances to reach the platform compared to control ones (P<0.01- P<0.001; Figure 3).

As Figure 4 shows, the total thiol concentration in cortical tissues of PTU group was significantly lower than that in control animals (Figure 4; P<0.001). The results also showed that cortical MDA concentration in PTU group was significantly higher than that in control group (Figure 5; P<0.001).

The total thiol concentration in hippocampal tissues of PTU group was significantly lower than control animals (Figure 6; P<0.001). MDA concentration in hippocampal tissues of PTU treated animals was higher than control group (Figure 7; P<0.001).

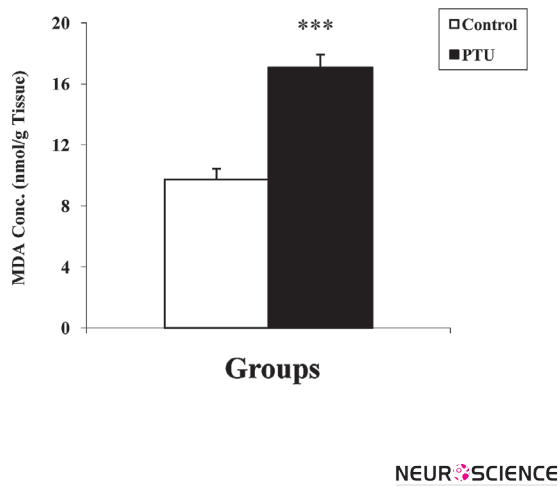

Figure 7. Comparison of MDA concentrations in hippocampal tissues between offspring of PTU and control groups. ***P<0.001 compared to control.

4. Discussion

In the present study, treatment of the rats during neonatal and juvenile periods by PTU impaired the spatial learning and memory. The results were consistent with our previous studies; however, methimazole was previously used to induce hypothyroididsm (Hosseini et al., 2010; Hosseini et al., 2012). It is suggested that PTU is preferred over methimazole during breast-feeding, because of its lower milk/serum concentration ratio and lower side effects (Momotani, 2006; Momotani et al., 2000).

Neonatal hypothyroidism and low levels of thyroid hormones during developmental periods is well known to be related to cognitive, learning and memory disturbances (Dugbartey, 1998; Haggerty et al., 1990; Kooistra et al., 1994; Smith, Evans, Costall & Smythe, 2002; Vara, Munoz-Cuevas & Colino, 2003; Kopp, Kitajima, & Jameson, 1996). Hypothyroidism impairs hippocampal-dependent learning and short- and long-term memory as well early and late phases of long-term potentiation (LTP) (Gerges & Alkadhi, 2004; Gerges, Alzoubi, Park, Diamond & Alkadhi, 2004; Alzoubi, Aleisa, Gerges& Alkadhi, 2006). The exact mechanisms which affect learning and memory and LTP have not been known. It is suggested that cognitive complications related to hypothyroidism are due to defects in brain development, inter-neuronal connectivity, and synaptic plasticity in the hippocampus (Rami, Rabie & Patel, 1986). The critical role of thyroid hormones for maturation and normal functions of the hippocampus has been well documented (Gerges & Alkadhi, 2004). It has been shown that hypothyroidism for even a short time, affects synaptic transmission and plasticity in the hippocampus (Dong et al., 2005; Gilbert & Paczkowski, 2003). It is also suggested that the effects of hypothyroidism are at least in part due to changes in the expression of some proteins such as in c-jun, c-fos and extracellular signal regulated kinases (ERKs) during crucial periods of brain development (Dong et al., 2005; Sui, Wang & Li, 2006). Yet, other proteins such as synapsin I, synaptotagmin I, and syntaxin which have a role in neurotransmitters' release are also affected by hypothyroidism (Vara, Martinez, Santos & Colino, 2002). It has also been suggested that thyroid hormone deficiency affects glutamate release (Hrabovszky, Turi, Kallo & Liposits, 2004; Shuaib et al., 1994; Sui, Anderson & Gilbert, 2005; Vara et al., 2002).

In addition, it is well known that neonatal hypothyroidism is accompanied by a delay in myelinogenesis and a decrease in the number of myelinated axons (Barradas, Ferraz, Ferreira, Daumas & Moura, 2000; Guadano Ferraz, Escobar del Rey, Morreale de Escobar, Innocenti, & Berbel, 1994). In the present study, it was shown that a deficiency of thyroid hormones during lactation and in the neonatal and juvenile periods could impair learning in the MWM test. The animals in the hypothyroid group had significantly higher time latency to find the platform during daily training. The findings of our previous study also showed an impairment in the MWM in adult rats with methimazole-induced hypothyroidism over a 180-day period (Hosseini et al., 2010). It has also been reported that treatment of thyroidectomized adult rats with thyroxine improved radial arm water maze tasks and LTP in the CA1 area of the hippocampus (Alzoubi, Gerges, Aleisa & Alkadhi, 2009). These researchers suggested that cyclic-AMP response element binding protein (CREB) and mitogen activated protein kinases (MAPKs) contribute to the impairment of hippocampal-dependent learning and memory (Alzoubi et al., 2009).

The association between nitric oxide (NO) and nervous system functions especially learning and memory has been widely investigated (Azizi-Malekabadi, Hosseini, Saffarzadeh, Karami & Khodabandehloo, 2011; Azizi-Malekabadi et al., 2012; Hosseini et al., 2010; Hosseini, Nemati Karimooy, Hadjzadeh & Safari, 2011; Hosseini, Sadeghnia, Salehabadi, Alavi & Gorji, 2009; Hosseini et al., 2011; Hosseini, Taiarani, Karami & Abad, 2011; Karami, Hosseini, Khodabandehloo, Khatami & Taiarani, 2011; Sadeghian, Fereidoni, Soukhtanloo, Azizi-Malekabadi & Hosseini, 2012). In our previous study, learning and memory impairment due to hypothyroidism during neonatal and juvenile period was accompanied by a high level of hippocampal NO metabolites (Hosseini et al., 2010). It has been previously reported that reac-

tive oxygen species (ROS) and lipid peroxidation was increased in the amygdala and hippocampus of rats after three weeks of treatment with methimazole which was accompanied with increased NO and elevated oxidative stress (Cano-Europa et al., 2008). Therefore, it was suggested that increased NO production in the hippocampus during hypothyroidism acts as an oxidative stressor. In the present study, it was shown that learning and memory impairments due to hypothyroidism during neonatal and juvenile growth were accompanied with brain tissues oxidative damage. In the present study, MDA concentrations in both hippocampal and cortical tissues were higher and total thiol groups were lower in PTU treated rats compared to control ones. The results of present study confirmed the other studies showing that hypothyroidism induces oxidative stress in the hippocampus (Cano-Europa et al., 2008). It has been also suggested that the antioxidant defense parameters of adult rat brain are considerably influenced by thyroid states of the body (Das & Chainy, 2004). The relationship between oxidative stress, neuronal damage and cognitive dysfunction has been well documented (Gustaw-Rothenberg et al., 2010; Head, 2009; Jellinger, 2009). It was also shown that oxidative stress and reactive oxygen species can cause learning and memory impairment (Behl & Moosmann, 2002; Abidin et al., 2004; Khodabandehloo, et al., 2013). Conversely, the antioxidants have been well shown to prevent the memory impairment in different experimental conditions (Silva et al., 2004).

Regarding these facts and the results of present study, a role for brain tissues oxidative damage as a possible mechanism of deleterious effects of PTU - induced hypothyroidism on learning and memory in neonatal and juvenile growth might be suggested.

Several studies confirmed the presence of oxidative stressors in hypothyroidism (Konukoglu et al., 2002; Yilmaz et al., 2003), but some other studies suggested that lipid peroxidation was decreased (Brzezinska-Slebodzinska, 2003) or unchanged (Das & Chainy, 2001). In a clinical trial, it was also found that the MDA, a marker of lipid peroxidation, was elevated in both hypothyroid and subclinical hypothyroid patients compared with the controls (Torun et al., 2009). It has been also suggested that thyroid hormones may play a role in direct regulation of expression of some antioxidant genes and other various genes associated with cell growth and proliferation (Miller et al., 2001; Santos et al., 2006). However, other studies did not show a significant change in levels of lipid peroxidation in the serum and thyroid tissues of hypothyroid rats. They showed that in all tissues of hypothyroid rats, the MDA levels did not differ significantly from euthyroid values (Petrulea et al., 2010; Venditti, Balestrieri, Di Meo & De Leo, 1997). In another study, it was shown that an overt hypothyroidism selectively increased the NO levels but did not modify the parameters of oxidative stress in the serum of fertile-age women (Coria, Pastran & Gimenez, 2009).

In conclusion the findings of this study imply that deleterious effects of hypothyroidism during neonatal and juvenile growth on learning and memory may at least in part be due to brain tissues oxidative damage.

Acknowledgment

The results described in this paper were from a M.D. student thesis proposal. The authors would like to thank the Vice Presidency of Research of Mashhad University of Medical Sciences for financial support.

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